

RESPONSE TO NON-FINAL OFFICE ACTION

U.S. Serial No.: 10/776,643

Filing Date: 12 February 2004

Title: Binding Proteins as Biosensors

Atty. Dkt. No. P-5430P1

AMENDMENTS TO THE SPECIFICATION

Please replace paragraph [0025], on page 7 as filed, with the following paragraph.

[0025] The term "Galactose/Glucose Binding Protein" or "GGBP" as used herein refers to a type of protein naturally found in the periplasmic compartment of bacteria. These proteins are naturally involved in chemotaxis and transport of small molecules (*e.g.*, sugars, amino acids, and small peptides) into the cytoplasm. GGBP is a single chain protein consisting of two globular α/β domains that are connected by three strands to form a hinge. The binding site is located in the cleft between the two domains. When glucose enters the binding site, GGBP undergoes a conformational change, centered at the hinge, which brings the two domains together and entraps glucose in the binding site. X-ray crystallographic structures have been determined for the closed form of GGBP from *E. coli* (N. K. Vyas, M. N. Vyas, F. A. Quiocho *Science* 1988, 242, 1290-1295) and *S. Typhimurium* (S. L. Mowbray, R. D. Smith, L. B. Cole *Receptor* 1990, 1, 41-54) and are available from the Protein Data Bank available on the world wide web at www.rcsb.org/pdb/ (~~http://www.rcsb.org/pdb/~~) as 2 GBP and 3 GBP, respectively. The wild type *E. coli* GGBP DNA and amino acid sequence can be found at www.ncbi.nlm.nih.gov/entrez/accession number D90885 (genomic clone) and accession number 230520 (amino acid sequence). Preferred GGBP is from *E. coli*.

Please replace paragraph [0027], on page 8 as filed, with the following paragraph.

[0027] Exemplary mutations of the *E. coli* GGBP protein include a cysteine substituted for a lysine at position 11 (K11C); a cysteine substituted for aspartic acid at position 14 (D14C); a cysteine substituted for valine at position 19 (V19C); a cysteine substituted for asparagine at position 43 (N43C); a cysteine substituted for glycine at position 74 (G74C); a cysteine substituted for tyrosine at position 107 (Y107C); a cysteine substituted for threonine at position

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110 (T110C); a cysteine substituted for serine at position 112 (S112C); a double mutant including a cysteine substituted for serine at position 112 and serine substituted for leucine at position 238 (S112C/L238S); a cysteine substituted for lysine at position 113 (K113C); a cysteine substituted for lysine at position 137 (K137C); a cysteine substituted for glutamic acid at position 149 (E149C); a double mutant including a cysteine substituted for glutamic acid at position 149 and a cysteine substituted for leucine at position 238 (E149C/L238C); a double mutant including a cysteine substituted for glutamic acid at position 149 and a serine substituted for leucine at position 238 (E149C/L238S); a double mutant including a serine substituted for alanine at position 213 and a cysteine substituted for histidine at position 152 (H152C/A213S); a cysteine substituted for methionine at position 182 (M182C); a cysteine substituted for alanine at position 213 (A213C); a double mutant including a cysteine substituted for alanine at position 213 and a cysteine substituted for leucine at position 238 (A213C/L238C); a cysteine substituted for methionine at position 216 (M216C); a cysteine substituted for aspartic acid at position 236 (D236C); a cysteine substituted for leucine at position 238 (L238C); a cysteine substituted for aspartic acid at position 287 (D287C); a cysteine substituted for arginine at position 292 (R292C); a cysteine substituted for a valine at position 296 (V296C); a triple mutant including a cysteine substituted for glutamic acid at position 149, an alanine substituted for serine at position 213 and a serine substituted for leucine at position 238 (E149C/A213S/L238S); a triple mutant including a cysteine substituted for glutamic acid at position 149, an arginine substituted for an alanine at position 213 and a serine substituted for leucine at position 238 (E149C/A213R/L238S); a triple mutant including a cysteine substituted for glutamic acid at position 149, a cysteine substituted for alanine at position 213 and a cysteine substituted for leucine at position 238 (E149C/A213C/L238C); a triple mutant including a cysteine substituted for glutamic acid at position 149, a cysteine substituted for alanine at position 213 and an asparagine for lysine at position 223 (E149C/A213C/K223N); a triple mutant including a cysteine substituted for glutamic acid at position 149, an asparagines for lysine at position 223 and an arginine for asparagine at position 256 (E149C/K223N/N256R); a triple mutant including a cysteine substituted for glutamic acid at position 149, an arginine substituted for alanine at

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position 213 and a cysteine substituted for leucine at position 238 (E149C/A213R/L238C); and in a particularly preferred embodiment, a triple mutant including a cysteine substituted for glutamic acid at position 149, a cysteine substituted for alanine at position 213 and a serine substituted for leucine at position 238 (E149C/A213C/L238S). Quadruple (and higher) mutants are also included, for example a mutant in which serine replaces alanine at position 1, cysteine replaces glutamic acid at position 149, arginine replaces alanine at position 213 and serine replaces leucine at position 238 (A1S/E149C/A213R/L238S); a mutant in which serine replaces alanine at position 1, cysteine replaces glutamic acid at position 149, serine replaces alanine at position 213 and serine replaces leucine at position 238 (A1S/E149C/A213S/L238S); and a mutant in which cysteine replaces glutamic acid at position 149, cysteine replaces methionine at position 182, cysteine replaces alanine at position 213 and serine replaces leucine at position 238 (E149C/M182C/A213C/L238S).